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REVIEW ARTICLE

Nanomaterial-assisted microfluidics for multiplex assays

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Abstract
Simultaneous detection of different biomarkers from a single specimen in a single test, allowing more rapid, efficient, and low-cost analysis, is of great significance for accurate diagnosis of disease and efficient monitoring of therapy. Recently, developments in microfabrication and nanotechnology have advanced the integration of nanomaterials in microfluidic devices toward multiplex assays of biomarkers, combining both the advantages of microfluidics and the unique properties of nanomaterials. In this review, we focus on the state of the art in multiplexed detection of biomarkers based on nanomaterial-assisted microfluidics. Following an overview of the typical microfluidic analytical techniques and the most commonly used nanomaterials for biochemistry analysis, we highlight in detail the nanomaterial-assisted microfluidic strategies for different biomarkers. These highly integrated platforms with minimum sample consumption, high sensitivity and specificity, low detection limit, enhanced signals, and reduced detection time have been extensively applied in various domains and show great potential in future point-of-care testing and clinical diagnostics.

Keywords Multiplexed detection · Biomarkers · Integrated-microfluidic devices · Nanomaterials · Point-of-care testing · Biochemical analysis

Introduction

Biomarkers are "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention," as defined by the National Institutes of Health Biomarkers Definitions Working Group in 2001 [1].

Biomarkers could provide valuable information regarding biological processes in the body, reflecting normal or abnormal physiological states. According to their functions, biomarkers can be classified into five categories: (i) antecedent biomarkers that evaluate the risk of developing a disease; (ii) screening biomarkers that identify individuals with subclinical disease; (iii) diagnostic biomarkers that help recognize overt disease; (iv) staging biomarkers that reveal the stage of disease; and (v) prognostic biomarkers that offer information on the outcome of a disease, monitor therapy efficiency, and provide evidence of recurrence [2–4]. Hence, monitoring biomarkers plays an essential role in clinical practice, molecular biology, and personalized medicine, especially for the early diagnosis of disease, tracking of disease progression, elucidation of disease mechanisms, prediction of treatment efficiency, and guidance of therapy.

Indeed, in many cases, the decision made based only on a single biomarker does not allow an accurate diagnosis of disease or efficient monitoring of therapy. Sequential assessment of multiple potential targets not only increases experimental time, cost, and the amount of data generated but also requires a large quantity of specimens that may be valuable or difficult to collect, for example, extremely rare circulating tumor cells (CTCs) in patient whole blood [5].

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Multiplex assays, referring to the simultaneous detection of multiple targets from a single specimen in a single test, are time-saving and cost-effective, and meet the challenge of limited sample analysis [6, 7]. Currently, three strategies are mainly used to realize multiplexing: (i) using spots or wells to spatially separate the detection sites; (ii) utilizing enzymes, dyes, or nanomaterials to label the reagents; and (iii) employing discrete zones of a channel network or electrode arrays to create separated regions [7].

With the innovation of nanotechnology and nanofabrication that allows miniaturization and incorporation of sophisticated analytical functions on a single platform, microfluidics has rapidly emerged as a promising technology in multiplex assays. Being capable of precisely manipulating small volumes of fluids (typically in the range of picoliters to nanoliters) and of realizing large-scale parallel analysis with reduced time [8, 9], microfluidic technologies possess numerous advantages for analytical chemistry, especially for multiplexed analysis of biomarkers in complex biological specimens, including high integration, high throughput, low sample and reagent consumption, easy portability, short turnaround time, low cost, etc. [10].

Nevertheless, only using microfluidic devices frequently encounters limitations in sensitivity and selectivity, which restricts their further employment for the assessment of complex clinical samples. The combination of microfluidics with nanomaterials preserving the advantages of both technologies has become a recent trend for ultrahigh-sensitivity detection. First, the nanoscale size allows them to liberally flow through the microchannels and to be easily confined in the microstructures, serving as efficient capturing or signaling agents, which may contribute to improving the detection sensitivity. Second nanomaterials possess remarkable unique characteristics, such as specific catalytic properties, extraordinary magnetic or optical properties, a high surface-to-volume ratio, and easy chemical functionalization, which could facilitate reactions, promote sample separation, favor signal transmission, serve as labels, etc., thus dramatically improving the analytical features of the microfluidic devices toward the improvement of sensitivity and specificity [11]. In brief, nanomaterial-assisted microfluidics offers new opportunities for more rapid, precise, and sensitive detection of biomarkers [12, 13].

In this review, we will give an overview of recent advances in nanomaterial-assisted microfluidic approaches for multiplexed detection and analysis of various biomarkers (Fig. 1). First, we briefly summarize the most commonly used microfluidic analytical approaches for multiplex assays, including microfluidic arrays, droplet microfluidics, microfluidic paper-based analytical devices, and microfluidic slip-driven devices. Next, a variety of nanomaterials that have contributed to improving the performance of microfluidic

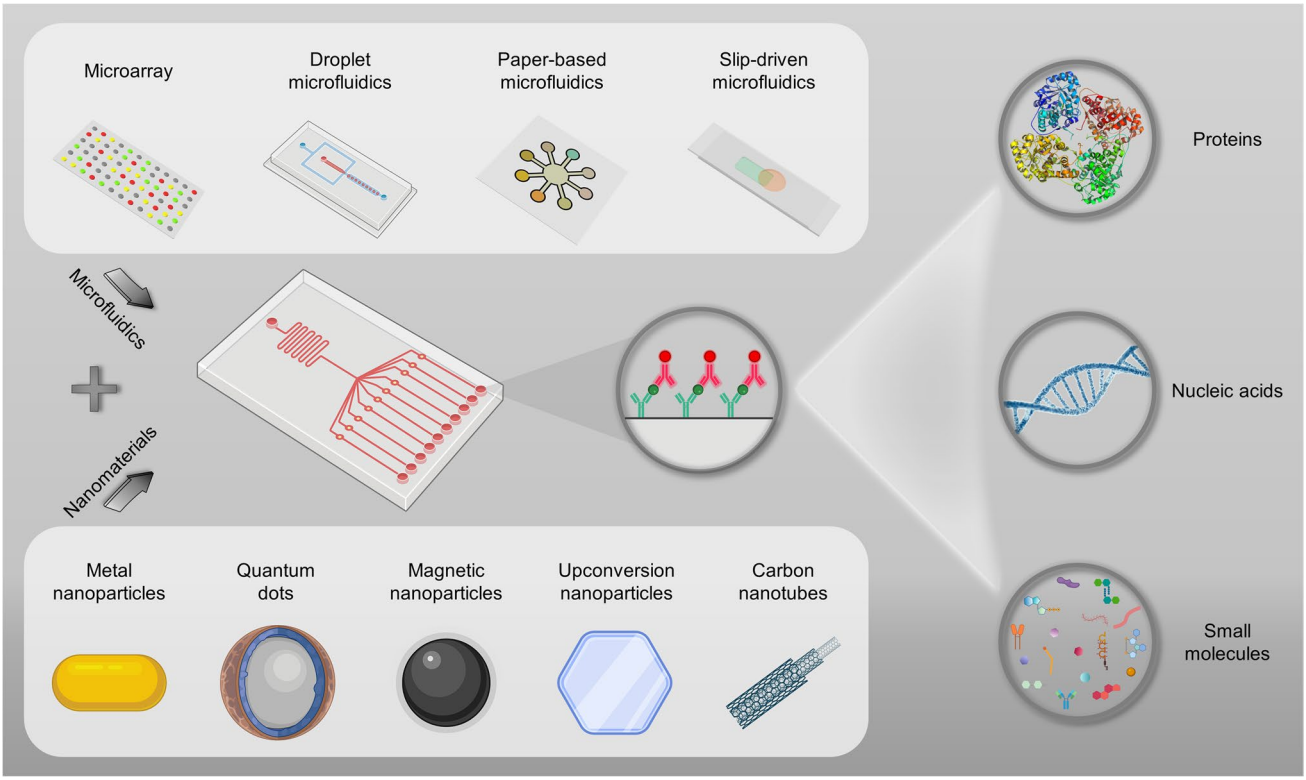


Fig. 1 Schematic illustration of multiplexed analysis of biomarkers using nanomaterial-assisted microfluidic devices

analytical systems are briefly presented, such as metal nanoparticles, quantum dots, magnetic nanoparticles, upconversion nanoparticles, and carbon nanotubes. Then, we focus on the utilization of nanomaterial-assisted microfluidic platforms for multiplexed analysis of biomarkers, including proteins, nucleic acids, and small molecules. For each type of biomarker, we highlight their characteristics, clinical significance, and typical detection strategies. Finally, we discuss some potential applications of nanomaterial-assisted microfluidics in disease diagnosis, environmental monitoring, and food safety control, followed by a brief summary and outlook. As the field of microfluidics is expanding rapidly, readers should be aware that this review is limited to the current state of knowledge.

Microfluidic devices for multiplex assays

Microfluidics refers to the manipulation of fluids at small scales (typically from 10^{-8} to 10^{-18} L) in narrow (10^{-6} to 10^{-8} m) channels or chambers [10] and is also termed a micro total analysis system (μTAS) or lab-on-a-chip (LOC). Recently, numerous microfluidic devices have been developed for multiplex assays based on different techniques. Herein, we will highlight several of the most commonly used classes, including microfluidic arrays, droplet microfluidics, microfluidic paper-based devices, and microfluidic slip-driven devices. Other microfluidic devices such as continuous flow chip [14, 15], microwell-based platform [16, 17], and self-powered microfluidic chip [18–20] have also been reported to be applied for multiplex assays and readers could refer to more complete descriptions elsewhere [21].

Microfluidic array

A microfluidic array, also termed a microarray, refers to an ordered arrangement of thousands of assay units in a single miniaturized device, which allows high-throughput and simultaneous analysis of a large number of biomolecules or parameters in a single test. This technique, which requires reduced consumption of samples and reagents, builds a simple, fast, and sensitive analytical technology.

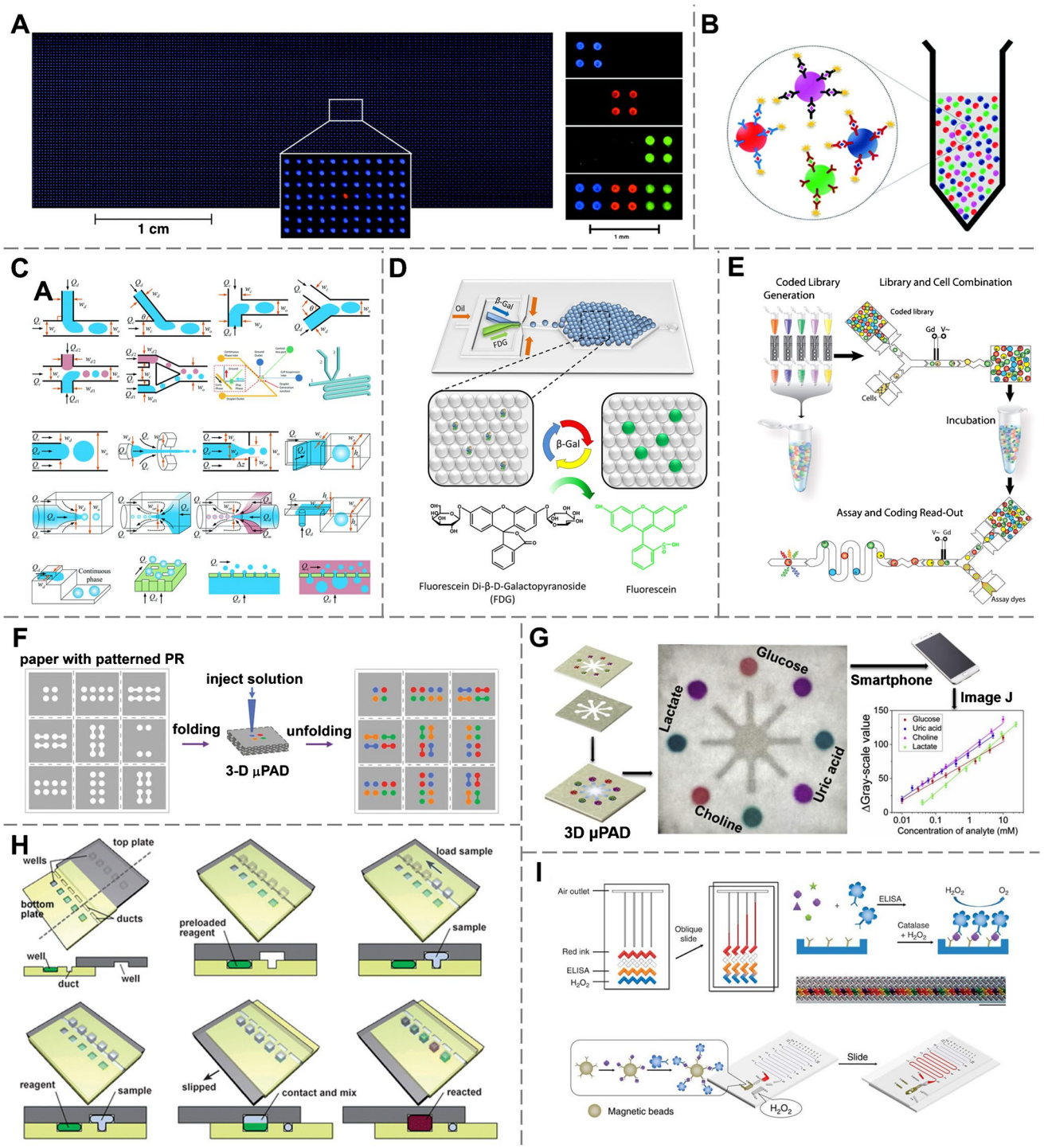
The two main categories of microfluidic arrays are protein microarrays aiming at protein–protein interaction analysis or protein profiling [22–24], and nucleic acid microarrays focusing on the qualitative or quantitative detection of nucleic acid sequences via hybridization [25]. Microarrays can also be classified according to their formats as follows: (i) two-dimensional planar arrays (Fig. 2A) based on dense microspots of ligands (such as protein, peptide, and aptamer) deposited onto a glass or other solid support [24] and (ii) three-dimensional suspension microsphere arrays (Fig. 2B) based on microspheres functionalized or coated with capture molecules to bind the specific analytes in a biological sample [26]. Both formats have respective characteristics. The target molecule needs to diffuse to the planar surface to ensure solid binding, which is less favorable than in the case of suspension arrays [27], while the microspheres should be labeled for facile recognition [28]. By fixing different capture agents at different zones of planar substrates or onto sphere surfaces, microarrays provide a fast and cost-effective way to simultaneously detect various proteins or genes and have been used in a wide range of application areas, such as biomarker discovery [29, 30], protein interaction studies [31], functional proteomic studies [22, 23], sequence analysis and genotyping [32, 33], drug discovery and development [34], and personalized medicine [35].

Droplet microfluidics

Unlike other continuous flow systems, droplet microfluidics involves the creation of discrete volumes (droplets) ranging from picoliters to nanoliters [43], using immiscible phases in microdevices. Droplet formation and manipulation (such as sorting, coalescence, splitting, mixing, and trapping) can be achieved using passive methods, including coflow, cross-flow and flow focusing, or active methods (with external forces), including electrical fields, magnetic fields, thermal control, mechanical forces, etc. [44, 36] (Fig. 2C). With the capacity to handle miniature fluid volumes, droplet microfluidics allows enhanced mixing and mass transfer, facilitating faster reactions. Moreover, each droplet is independent and can be individually transported, mixed, and analyzed, allowing parallel and high-throughput analysis [45].

The typical protocol for bioassays in droplet microfluidics mainly consists of reagent encapsulation in droplets, initiation of reaction by mixing or coalescence of liquids, and signal readout [37] (Fig. 2D). It should also be noted that droplet indexing (Fig. 2E), for example, by encapsulating fluorescent dyes [38], nanomaterials possessing particular optical properties [46, 47] or DNA-barcoded microbeads [48] into droplets, or by directly barcoding the droplet contents with DNA oligonucleotide before encapsulation [48, 49], is crucial for exact differentiation between droplets involving various reagents for multiplex assays.

With remarkable advantages, including miniaturization, compartmentalization, monodispersity, parallelization and high throughput, droplet microfluidics for biochemical analysis has now been widely applied in DNA identification and sequencing, protein–protein interaction studies, immunoassays, clinical diagnosis, drug screening, environmental monitoring, etc. [50].



Microfluidic paper-based analytical device

A microfluidic paper-based analytical device, abbreviated as "μPAD," refers to a microfluidic device made of paper or other porous membranes and is fabricated by either shaping/cutting the paper to create microfluidic channels or patterning hydrophobic barriers on the substrate via various techniques such as photolithography and wax printing [51–54]. Paper folding (origami) is also a popular way to fabricate a three-dimensional μPAD (Fig. 2F) [39]. The first μPAD was developed by Martinez et al. [55] in 2007 using chromatography paper patterned with SU-8 photoresist via photolithography. By guiding reagents to appropriate areas, this device was suitable for measuring multiple targets in parallel (Fig. 2G) [40]. Using porous paper as the substrate, fluids are generated and driven by capillary forces, with the

◂**Fig. 2** Commonly used microfluidic devices for multiplexed detection of biomarkers. (A) Planar microarray holding 10,800 spots for protein–protein interaction detection. Reprinted with permission from [24]. Copyright 2000, American Association for the Advancement of Science. (B) Suspension array of encoded microspheres. Reprinted with permission from [28]. Copyright 2014, Royal Society of Chemistry. (C) Different methods for droplet formation. Reprinted with permission from [36]. Copyright 2017, Royal Society of Chemistry. (D) Schematic illustration of the droplet analysis protocol, including reagent encapsulation, reaction, and signal release. Reprinted with permission from [37]. Copyright 2014, American Institute of Physics. (E) Optical encoding in microfluidic droplets. Reprinted with permission from [38]. Copyright 2019, National Academy of Sciences. (F) 3D μPAD fabricated by the origami-based method. Reprinted with permission from [39]. Copyright 2011, American Chemistry Society. (G) Simultaneous detection of glucose, uric acid, lactate, and choline by a double-layered μPAD using multiple colorimetric indicators as readouts. Reprinted with permission from [40]. Copyright 2019, Elsevier. (H) Composition and working principle of SlipChip. Reprinted with permission from [41]. Copyright 2009, Royal Society of Chemistry. (I) Volumetric measurement and direct colorimetric readout in the V-chip. Reprinted with permission from [42]. Copyright 2012, Nature Publishing Group

flow speed controlled by the porosity and the flow direction defined by the hydrophobic barrier geometry. Without the need for external equipment, this technique is simple, cost-effective, portable, easy to use, and easy to dispose, and offers the possibility of multivariate detection [56].

Combined with various signal readout strategies, such as optical microscopy, electrochemistry, chemiluminescence, and electrochemiluminescence, μPAD has been extensively employed for the analysis of various biomolecules, including glucose uric acid (UA), lactic acid, nitrite, cholesterol, proteins, RNA, and DNA [51, 57]. Possessing unique properties such as portability, low cost, and easy disposal, μPAD offers advantageous solutions for point-of-care testing (POCT) [58, 59], food contamination control [60], environmental monitoring [61], medical diagnosis [62], etc.

Microfluidic slip-driven device

A microfluidic slip-driven device consists of two or more plates that can be moved relative to one another by slipping motion to manipulate fluids. The wells or ducts with preloaded or user-loaded reagents and samples will be connected or disconnected through the relative movement of plates, generating reactions for sample detection [63, 64].

Since the report of the first slip-driven device, named SlipChip (Fig. 2H), in 2009 [41], numerous variants have been developed based on different materials [65–69], different mechanisms of motion [70–73], or different readout strategies [72, 74–75]. The most famous forms include SlipPAD, Slipdisc, and volumetric bar-chart chip (V-Chip). SlipPAD is a paper analytical device based on the SlipChip concept composed of two chromatographic paper layers patterned by printing wax attached to rigid substrates, bringing advantages of robustness and high throughput to the inherent simplicity of paper-based microfluidics [68, 76]. Slipdisc is a rotational slip-driven chip working based on a clockwork mechanism, which allows accurate and controlled movement of every step in an assay [72]. V-Chip uses an ink-based bar chart for volumetric measurement of oxygen production during the chemical reaction (Fig. 2I), allowing direct and visualized readout of results without using external instruments [42].

Without the need for external pumps, valves, or other complex instruments and capable of performing multistep and multiplexed analysis by simple slip manipulation, slip-driven devices have become an attractive platform in a large number of studies, including nucleic acid analysis [77], protein crystallization [78], immunoassays [79], and point-of-care diagnostics of variant biomarkers [80].

Nanomaterials integrated into microfluidic devices

A nanomaterial, defined by the European Commission, is "a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1–100 nm" [81]. A common categorization of nanomaterials is according to the number of sizes beyond the nanometer scale, yielding 0-D nanomaterials (nanoparticles, nanospheres, nanodots), 1-D nanomaterials (nanofibers, nanowires, nanorods, nanotubes), 2-D nanomaterials (nanolayers), and 3-D nanomaterials (nanocomposites) [82].

The integration of nanomaterials in microfluidics benefits greatly from the distinct properties of nanomaterials and could significantly improve the performances of microfluidic devices [11] in the multiplexed analysis of biomarkers, especially in the following aspects: (i) more efficient target preconcentration and separation by nanomaterials taking advantage of their large surface area-to-volume ratio and relatively easy functionalization; (ii) more rapid and facile analytical reactions thanks to nanomaterials acting as catalysts or electron-transfer mediators; (iii) enhanced signal readout and increased detection sensitivity owing to the unique electrical, optical, or plasmonic properties of nanomaterials; and (iv) improved multiplexity profiting from the significant encoding capacity of several nanomaterial types.

Herein, we discuss some of the most popular biocompatible nanomaterials suitable for biomarker analysis, including metal nanoparticles, quantum dots, magnetic nanoparticles, upconversion nanoparticles, and carbon nanotubes, along with their functions in microfluidic assays.

Many other nanomaterials, including but not limited to graphene oxide, silicon nanowires, silicon nanopillars, and gold nanosheets, have also been reported to be applied in microfluidic multiplex assays due to their specific properties, and readers can refer to other works [83, 84] for more complete comprehension.

Metal nanoparticles

Metal nanoparticles are nanosized metals with dimensions ranging from 10 to 100 nm. Their remarkable properties make them the most popular candidate along with microfluidic analytical systems.

Nobel metals such as gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs) possess localized surface plasmon resonance (LSPR), i.e., the resonance oscillation occurring on the surface and their absorption in the ultraviolet–visible range is dependent on particle sizes [85]. Therefore, modification of size, shape, and aggregation degree results in a color change, which could be used as labeling or colorimetric signals. In addition, modification or functionalization of metal nanoparticles could amplify the LSPR for signal enhancement [86] in microfluidic analytical systems (Fig. 3A).

Due to the favorable plasmonic response, metal nanoparticles can also enhance the electromagnetic field around Raman reporters to realize surface-enhanced Raman scattering (SERS) [91, 92]. For example, SERS nanotags are generally composed of a metal colloid with a Raman reporter protected within a shell and have been applied in the simultaneous detection of protein biomarkers in microfluidic devices [93–95].

Furthermore, the excellent catalytic performance endows metal nanoparticles with another important ability, i.e., acting as a catalyst to promote reactions, which helps improve the detection sensitivity [96, 97].

Quantum dots

Quantum dots (QDs) are semiconductor core–shell structured nanoparticles based on II-VI, III-V, I-III-V compounds or perovskites [98]. QDs have unique optical properties, including broad excitation, narrow emission, tunable emission wavelength, long fluorescence lifetime, high photoluminescence (PL), and photochemical stability. Specifically, QDs of different sizes excited at a single wavelength can emit at different wavelengths. This property is known as the quantum size effect (Fig. 3B) [87], making QDs ideal fluorescent labels [99] contributing to a dramatic improvement

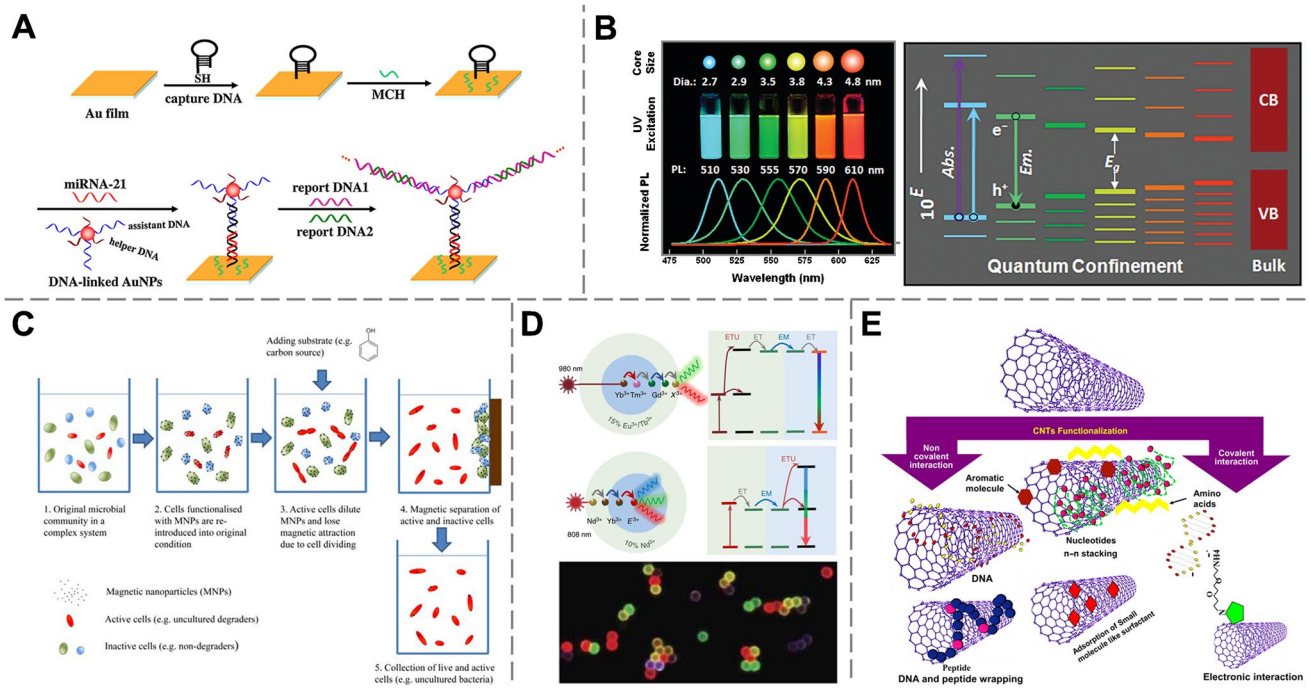


Fig. 3 Nanomaterials commonly used in biomarker analysis. (A) Gold nanoparticles enhancing surface plasmon resonance. Reprinted with permission from [86]. Copyright 2016, Elsevier. (B) Quantum size effect of quantum dots. Reprinted with permission from [87]. Copyright 2011, American Chemistry Society. (C) Magnetic nanoparticles contributing to cell isolation. Reprinted with permission from [88]. Copyright 2015, Nature Publishing Group. (D) Energy migration within upconversion nanoparticles. Reprinted with permission from [89]. Copyright 2018, Nature Publishing Group. (E) Colvant and noncolvant functionalization of carbon nanotubes. Reprinted with permission from [90]. Copyright 2019, Elsevier

of detection multiplexity [100] in microfluidic analytical devices [46, 47] for a variety of applications [101].

The theoretical coding capacity of QDs is determined according to the formula $N^m - 1$, where N is the number of intensity levels and m is the number of emission colors in the encoding system [102]. This is particularly advantageous over traditional fluorescent dyes whose encoding capacity is the product of the number of dyes times the number of dye concentrations [103]. Nevertheless, despite advances in fluorescence microscopy development, only six fluorescent colors can be distinguished [104], and the practical encoding capacity is further limited to approximately 100 by the spectral overlap [105] and energy transfer [47, 106].

Magnetic nanoparticles

Magnetic nanoparticles (MNPs) are nanoparticles displaying superparamagnetic properties. They could be iron oxide (maghemite γ-Fe_2O_3 or magnetite Fe_3O_4), alloys (FePt), or pure metals (Fe and Co). MNPs show remarkable properties [107], such as high saturation magnetization, high field irreversibility, extra anisotropy contributions, and shifted loops after field cooling. Indeed, MNPs can be manipulated by applying an external magnetic field and are easily recovered when the magnetic field is removed. This unique property makes MNPs ideal support for target separation (Fig. 3C) [108, 109] when they are conjugated with selective agents such as enzymes and antibodies, which allows for simple, rapid, efficient, and reversible target isolation and recognition. MNPs are easily decorated with other nanomaterials, organic or inorganic coatings, and biomolecules to attain biocompatibility and to enhance the separation efficiency [110, 88]. The integration of functionalized MNPs in microfluidics has been demonstrated to improve the sensitivity and selectivity of biomarker detection [111, 112].

Upconversion nanoparticles

Upconversion nanoparticles (UCNPs) are lanthanide-doped rare-earth-element luminescent materials that exhibit an anti-stoker shift (upconversion luminescence), i.e., emitting a photon with higher energy (shorter wavelength) than the absorbed one [113, 114]. That is, UCNPs could be excited with near-infrared radiation and emit visible radiation (Fig. 3D), making them hold multiple outstanding photophysical properties. Near-infrared excitation permits a large penetration depth in samples and decreases the autofluorescence, resulting in a strong signal-to-background ratio, which could help improve the detection sensitivity of the analytical devices.

The photoluminescence of UCNPs arises from the 4f electron transitions, and the optical properties (including colors) of UCNPs are tunable through variation of lanthanide dopants, for example, by changing ion type and composition [89, 115]. Therefore, UCNPs are also extraordinary optical labeling nanomaterials with a great encoding capacity (n intensity levels with m colors could generate $n^m - 1$ unique codes) [116] and display good performance in the multiplexed analysis of proteins [117]. Labeling using UCNPs is even more convenient, straightforward, and less limited than QDs since UCNPs emit light in the visible wavelength range.

Carbon nanotubes

Carbon nanotubes (CNTs) are cylindrical fullerenes consisting of "rolled-up" graphitic sheets with an approximate inner diameter on the nanometer scale and were first discovered in 1991 by Iijima during fullerene synthesis [118]. CNTs can be classified into three types according to the number of concentric tubes: single-walled carbon nanotubes (SWCNTs), double-walled carbon nanotubes (DWNTs), and multi-walled carbon nanotubes (MWNTs) [119]. Their unique properties, including a high aspect ratio, chemical stability and outstanding mechanical strength make CNTs ideal supports for a wide variety of molecules such as nucleotides, amino acids, surfactant molecules, DNA, antibodies and enzymes through either covalent or noncovalent functionalization (Fig. 3E) [90]. The large surface area permitted attachment of more abundant reagents or targets, thus can enhance the output signals and improve the detection sensitivity. In addition, they possess excellent electrical and thermal conductivity, allowing for electrochemical sensing [120, 121]. CNTs are widely used in microfluidic devices for the detection of proteins [122, 123], nucleic acids [120, 121], and other molecules [124], with improved detection sensitivity.

Multiplexed detection of biomarkers by nanomaterial-assisted microfluidic platforms

Biomarkers could be physical traits such as blood glucose level, physiologic measurements such as blood pressure, and imaging tests such as echocardiogram [2], but currently they are more typically referred to as traits of biochemical molecules (proteins, genomics, cholesterol, glucose, etc.) [3, 125]. In this section, we classify three important groups of molecular biomarkers, i.e., proteins, nucleic acids and small molecules, and give a brief overview of their characteristics and detection methodologies by highlighting their multiplexed measurement using nanomaterial-assisted microfluidic techniques.

Proteins

Proteins are important biological macromolecules involved in numerous biological functions, including protection against viruses, providing structure and support for cells, transmitting signals, and catalyzing reactions. Many diseases are associated with abnormal protein expression, e.g., Alzheimer's disease, cardiovascular disease, and cancer [126–128]. Thus, protein biomarker investigation in a complex biological sample could provide the basis for early disease diagnosis, novel therapeutic methodology intervention, new pharmaceutical development, etc.

Protein studies may include structural characterization of known purified proteins or protein–protein interactions, and qualitative or quantitative detection of target proteins in a biological sample, with the latter being emphasized in the following. Numerous techniques have been developed and applied for protein detection and can be roughly classified into three categories according to different detection fundamentals. (i) Immunoassay based on antibody-antigen affinity: An antibody, also named immunoglobulin, is a protein component of the immune system that can bind to a specific antigen. This specific chemical affinity between antibodies and antigens is called immunoaffinity, allowing highly selective recognition of proteins. The test measuring the presence or concentration of an analyte based on the immunoaffinity is termed "immunoassay," which is now the most commonly used protein analysis strategy [129–131]. Currently, enzyme-linked immunosorbent assay (ELISA) [132, 133], enzyme-linked immunospot (ELISPOT) [134], and western blot [135, 136] are among the most popular immunoassays, and ELISA is regarded as the gold standard tool for protein detection and quantification. (ii) Aptamer-based assay: aptamers are artificial, single-stranded DNA or RNA oligonucleotides selected in vitro using the SELEX (selective evolution of ligands by exponential enrichment) process from random-sequence nucleic acid libraries. Aptamers can bind to proteins with high affinity and specificity, thus giving rise to aptamer-based methods, such as enzyme-linked aptamer assays (ELAAs) [137], for protein detection. (iii) Mass spectrometry [138]: mass spectrometry can be used to determine the component of an unknown sample via measurement of the mass-to-charge ratio of ions. During this experiment, proteins are digested into peptides, which are then separated, fragmented, ionized, and analyzed by the mass spectrometer to generate peaks corresponding to each peptide fragment ion. Proteins are finally identified from the peaks of the captured mass spectra.

These techniques favor protein detection with high selectivity; however, challenges remain. First, a biological sample always contains many other interfering molecules, which increases the complexity for selective detection of the target protein. Second, some target proteins may be of low concentrations and thus difficult to detect, especially in the presence of abundant potentially interfering molecules. Third, concentrations of different proteins in a sample may differ by many orders of magnitude, limiting the multiplex assays.

To date, new and emerging techniques have been developed to address these challenges. Nanomaterial-assisted microfluidics may be an ideal solution due to the inherent superiorities of microfluidic techniques, such as high throughput, parallelization, portability, fast reaction time, and reduced reagent consumption. In addition, the integration of various nanomaterials contributes to the increase of multiplexity, improvement of detection sensitivity, and enhancement of signals.

QDs, possessing a great encoding capacity [139], have been demonstrated to encode microbeads as labels in microarray platforms to achieve multiplex assays, for example, for the simultaneous detection of anti-IgG and anti-IgM (Fig. 4A) [140]. Compared with the commercially available Luminex® xMAP™ technology utilizing microspheres (Luminex beads) stained with different proportions of red and infrared fluorophores[141] which also showed successful application for multiplexed detection of proteins in a microfluidic device [142], QD-encoded microbeads have drastically more coding possibilities. Incorporation of encoded microbeads or microspheres into microfluidics significantly improves the capability of performing multiplexed analysis [143–145].

The integration of biomolecule-functionalized nanomaterials, especially antibody-functionalized nanomaterials, has been proven to be a practical solution to improve the detection sensitivity, as they could serve as an amplified capture in a sandwich immunoassay and thus enhance the capture efficiency of target proteins. In particular, horseradish peroxidase (HRP), and capture antibody-functionalized AuNPs integrated into a microbead-based microfluidic array platform have been reported to greatly improve the detection sensitivity of the cancer biomarker α-fetoprotein (AFP) down to 0.2 pg/mL [150]. Antibody-conjugated SWCNTs have also been used as capture agents, along with labeled antibody-decorated silica nanoparticles as detection agents, to help realize a sandwich immunoassay in a 3D microarray. The device succeeded in ultrasensitive detection of eight proteins with limits of detection down to 78–110 fg/mL in diluted serum [151]. Another strategy has been proposed in μPAD where antibody-functionalized AuNPs as amplified capture agents and signal probes (Fig. 4B) help the platform simultaneously determine three acute myocardial infarction (AMI) biomarkers, i.e., heart-type fatty acid-binding protein (FABP), cardiac troponin I (cTnI) and copeptin, with extremely low limits of detection of 0.06, 0.3, and 0.4 pg/mL, respectively [146].

The combination of nanomaterials in microfluidic devices could also enhance the generated signals, for example, by employing metal nanomaterial SERS tags, nanowires, or nanotubes of a high aspect ratio. A SERS-assisted 3D microfluidic platform was developed using gold@silver core–shell

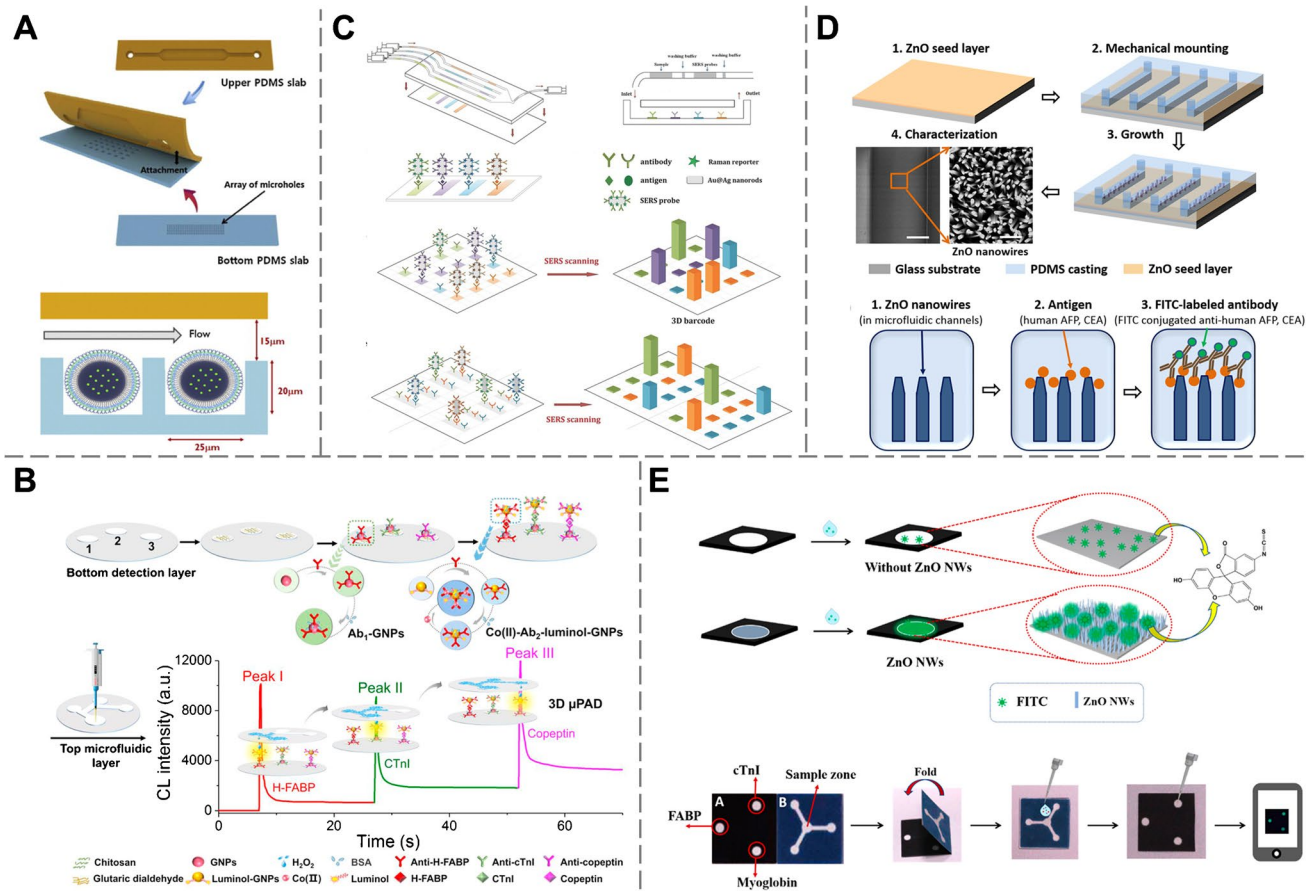


Fig. 4 Multiplexed detection of proteins using nanomaterial-assisted microfluidic devices. (A) Simultaneous analysis of anti-IgG and anti-IgM by planar microarray integrated with antibody-functionalized QDs. Reprinted with permission from [140]. Copyright 2015, Elsevier. (B) Dual-signal amplification strategy by functionalized nanoparticles in 3D μPAD. Reprinted with permission from [146]. Copyright 2020, Elsevier. (C) SERS nanotag-assisted 3D microfluidic chip allowing quantitative multiplex assay for proteins. Reprinted with permission from [147]. Copyright 2015, Wiley. (D) Immunoassay for protein detection in microfluidic channels integrated with ZnO NWs. Reprinted with permission from [148], Copyright 2018, Elsevier. (E) Detection of three cardiac biomarkers in ZnO NW-integrated μPAD with enhanced fluorescence signals. Reprinted with permission from [149]. Copyright 2019, American Chemistry Society

nanorods as SERS tags, where 2D spatial arrays help to differentiate target protein analytes and SERS spectroscopic information allows quantitative and simultaneous analysis of target proteins (Fig. 4C). This platform achieved simultaneous detection of six different antibodies with a detection sensitivity as low as 10 fg/mL [147]. Zinc oxide nanowires (ZnO NWs) have also been reported to enhance the fluorescence intensity in microfluidic chips (Fig. 4D), allowing for the detection of proteins with high sensitivity [148]. A fivefold enhancement of fluorescence intensities (Fig. 4E) was achieved in a paper-based fluorogenic immunodevice integrated with ZnO NWs for the detection of three cardiac biomarkers, FABP, cTnI, and myoglobin [149].

Nucleic acids

Nucleic acids are another classification of biological macromolecules and consist deoxyribonucleic acid (DNA), which is an ideal carrier of genetic information, and ribonucleic acid (RNA), which serves as the first intermediate in the conversion of the genetic information encoded in DNA to direct protein fabrication. Nucleic acids play an essential role in cell functioning and life continuity and are commonly used as biomarkers for cancer and other diseases such as neurodegenerative [152] and infectious diseases [153]. Specifically, evaluation of increased levels of circulating nucleic acids shed by apoptotic and necrotic cells into biological fluids is becoming an emerging diagnostic technique for low-cost and noninvasive cancer detection [154, 155]. Rapid and efficient nucleic acid detection plays a crucial role in many clinical applications, such as disease diagnostics, severity assessment, and treatment selection.

The detection of nucleic acids essentially uncovers their nucleotide sequence that is unique and can be considered as its identity. Nucleic acid detection can be achieved either by hybridization or by sequencing. (i) The hybridization-based

method involves the hybridization of a labeled probe complementary to the target sequence followed by detection of the label. Numerous methods have been developed corresponding to this fundamental, including Northern blot, Southern blot, quantitative PCR (qPCR), and real-time PCR, with the latter two the gold standard tools for nucleic acid analysis. (ii) Sequencing-based methods involve the determination of the order of nucleotides within the nucleic acid for identification, including Sanger sequencing, pyrosequencing, and next-generation sequencing (NGS) [156]. NGS, enabling high-throughput sequencing of nucleotide at a relatively low cost, is one of the most extensively employed technologies in fundamental research.

Challenges in nucleic acid detection mainly arise from the following reasons: (i) biological specimens may contain abundant interfering molecules such as proteins, carbohydrates, or other types of nucleic acids, further complicating the detection of target DNAs or RNAs; (ii) both intracellular and extracellular concentrations of DNAs and RNAs are extremely low, requiring the development of methods with a very low limit of detection; and (iii) there are approximately three billion nucleotides in the human genome; thus, the throughput and multiplexity of the detection method are essential to reduce testing time and cost.

To meet these challenges, three key processes are essential in a typical nucleic acid detection protocol: sample preparation, target amplification, and signal readout [157]. First, nucleic acids are isolated from the complex mixture to eliminate the interference of other molecules, e.g., by using magnetic particles [108]. Second, amplification is essential because the extremely low concentration of nucleic acids is insufficient for direct detection. Amplification could greatly improve the specificity and sensitivity of target detection. The most relevant amplification methods in diagnostics include rolling circle amplification (RCA), polymerase chain reaction (PCR), strand displacement amplification (SDA), loop-mediated isothermal amplification (LAMP), and nucleic acid sequence-based amplification (NASBA). Finally, the signal readout is normally realized by traditional laboratory-based methods, such as chemiluminescence, electrochemiluminescence, and fluorescence intensity.

Nevertheless, traditional detection techniques still suffer from long preparation and analysis times, limited specificity and sensitivity, sophisticated operation procedures, and complex external equipment, which limits the effectiveness of these methods in POCT. Nanomaterial-assisted microfluidics is a promising tool in nucleic acid detection for increasing multiplexity, improving detection sensitivity, and enhancing readout signals by taking advantage of the unique properties of different nanomaterials. Nucleic acid microarrays, droplet microfluidics, and paper-based microfluidic devices are the main microfluidic techniques employed here.

A variety of nanomaterials (such as QDs, UCNPs, and AuNPs) have been integrated into DNA microarrays to achieve multiplexed assays. Similar to its function in protein detection, QD-encoded microbeads are ideal candidates for the simultaneous detection of nucleic acids [158]. A QD-encoded microbead-based array implementation was reported to successfully identify three miRNA biomarkers (miRNA-21, miRNA-221, and miRNA-16) [159]. Encapsulating multicolored QDs into droplets as fluorescent labels allowed for multiplexed detection of miRNAs based on a microfluidic droplet chip [160]. The combined use of one single type of UCNP and three different kinds of QDs in a μPAD has been successfully employed for the simultaneous detection of three gene fragments (uidA, Stx1A and tetA) from serum samples [161]. AuNPs, benefiting from a high degree of polyvalency and plasmonic properties, have been reported to display an advantageous capacity of multiplexity [162]. Multiplexed DNA detection has been achieved on a spotted array chip based on a sandwich assay process with the help of barcoded DNA-functionalized AuNPs (Fig. 5A) [163].

Similar to their role in microfluidic protein detection, biomolecule-functionalized AuNPs have also been employed to improve nucleic acid detection sensitivity in DNA microarrays. HRP and DNA-functionalized AuNPs integrated into a bead-based microarray device (Fig. 5B) were demonstrated for sensitive genotyping of human papillomavirus DNA with a threefold detection sensitivity [164]. Another competitive DNA microarray platform was proposed by using single-strand DNA (ssDNA)-conjugated AuNPs to optimize hybridization competition. The device showed excellent performance in multiplexed detection of eight miRNAs, reaching a limit of detection not higher than 0.8 pM [166]. In addition, other nanomaterials and techniques have also shown a capacity for ultrasensitive multiplexed nucleic acid detection. Due to their fluorescence resonance energy transfer (FRET) sensing ability and enzyme protection capability, molybdenum disulfide (MoS_2) nanosheets have been applied in a droplet microarray for simultaneous detection of five viral nucleic acids (Fig. 5C) with limits of detection down to 1.24 and 1.26 nM for HIV-1 and HIV-2 genes, respectively [165].

Apart from the formation of core–shell-structured SERS tags, other modifications on metal nanoparticles could also result in signal amplification [167]. For example, surface roughness-controlled AuNPs were employed to significantly enhance SERS and contribute to the development of an ultrasensitive and reliable SERS sensor for the simultaneous detection of three microRNA biomarkers that are related to liver cancer: miRNA-21, miRNA-122, and miRNA-223 [168].

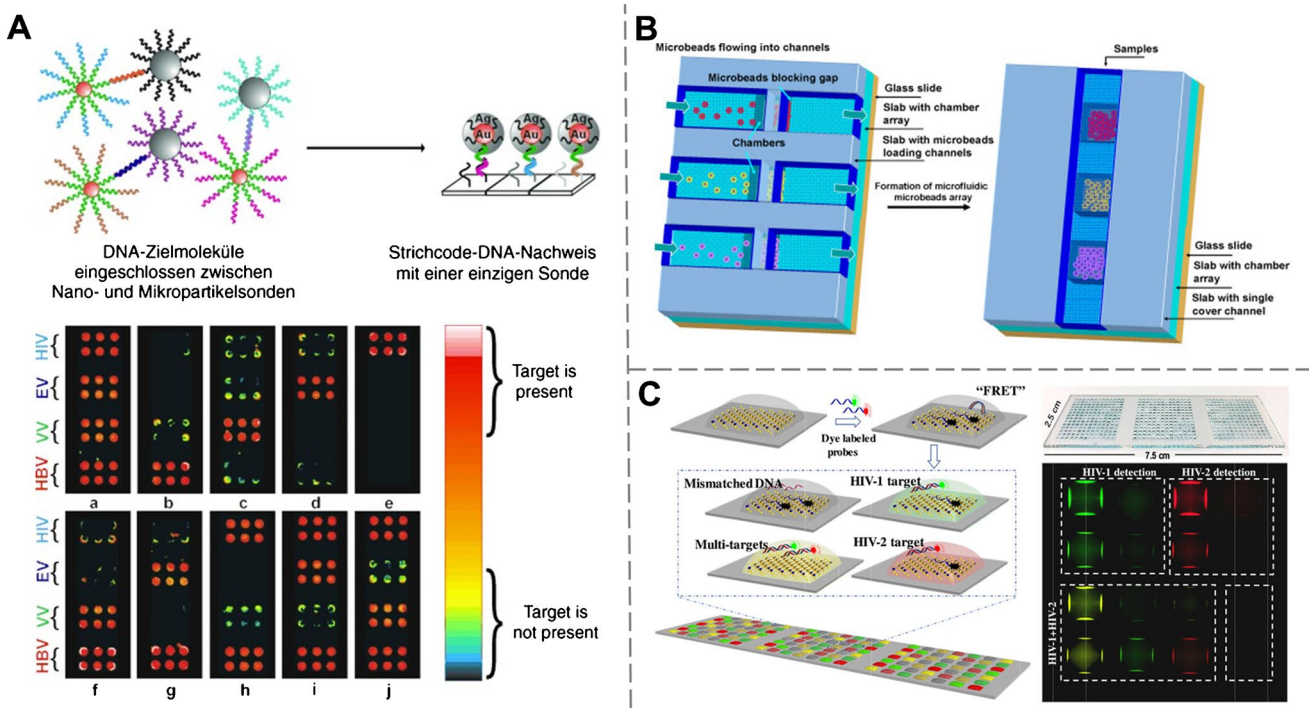


Fig. 5 Detection of multiple nucleic acids based on nanomaterial-assisted microfluidic devices. (A) A spotted array chip combined with DNA-functionalized AuNPs for multiplexed detection of DNA. Reprinted with permission from [163]. Copyright 2006, Wiley. (B) HRP-AuNPs-DNA conjugates in a bead-based microarray device assisting DNA detection. Reprinted with permission from [164]. Copyright 2011, Elsevier. (C) Droplet microarray platform modified with MoS_2 for multiplexed detection of five viral nucleic acids. Reprinted with permission from [165]. Copyright 2020, American Chemical Society

Small molecules

Proteins and nucleic acids represent the two classes of macromolecular biomarkers, and small molecular biomarkers are also of great value and deserve deep investigation. These small molecules may be intermediates or products derived from metabolic processes and can be found either locally in the tissue where they are produced or systematically in different biofluids, such as blood, urine, saliva, and cerebral spinal fluid. The two types of small molecules most frequently used in the clinical diagnosis of common diseases are lipids (LDL cholesterol, HDL cholesterol, triglycerides, phospholipids, prostaglandins, etc.) and carbohydrates (glucose, glycan, sucrose, etc.). Others may include but are not limited to reactive oxygen species such as H_2O_2, uric acid, lactate, urea, and creatinine. Changes in small molecular biomarker concentrations can provide information on biological processes that are strongly related to diseases and therapeutic responses, for example, cholesterol and triacylglycerol for cardiovascular disease [169], blood glucose and hemoglobin A1c for diabetes, neurofilaments for neurological disorders [170], and brain natriuretic peptide (BNP) for heart failure [171]. The detection of small molecular biomarkers shows great significance in the clinical diagnosis of disease, evaluation of therapeutic efficiency, prognosis, etc.

Since small molecular biomarkers contain a large number of substances with particular biological and chemical properties, their detection fundamentals and approaches vary from one substance to another. Here, we highlight several interesting nanomaterial-assisted microfluidics implementations for the simultaneous detection of small molecule biomarkers, commonly developed based on droplet microfluidics, paper-based chips, and slip-driven devices. More complete overviews of these molecular biomarker detection methods can be found elsewhere [172].

Multiplexity in nanomaterial-assisted microfluidics is frequently satisfied using nanomaterials with particular optical properties. For example, the optical properties of AuNPs on paper change with their aggregation degrees and hydrophobic properties influenced by the analyte concentrations. A novel plasmonic AuNP-based 3D paper microfluidic platform was pioneered for colorimetric assays without utilizing enzymes or any chromogenic substrates (Fig. 6A). This device showed success in the multiplexed detection of glucose, uric acid, and free cholesterol [173].

The employment of ZnO nanomaterials in μPADs could provide higher detection sensitivity due to their high surface-to-volume ratio and high enzyme-capturing efficiency. Successful integration of ZnO nanowires [175] in μPADs

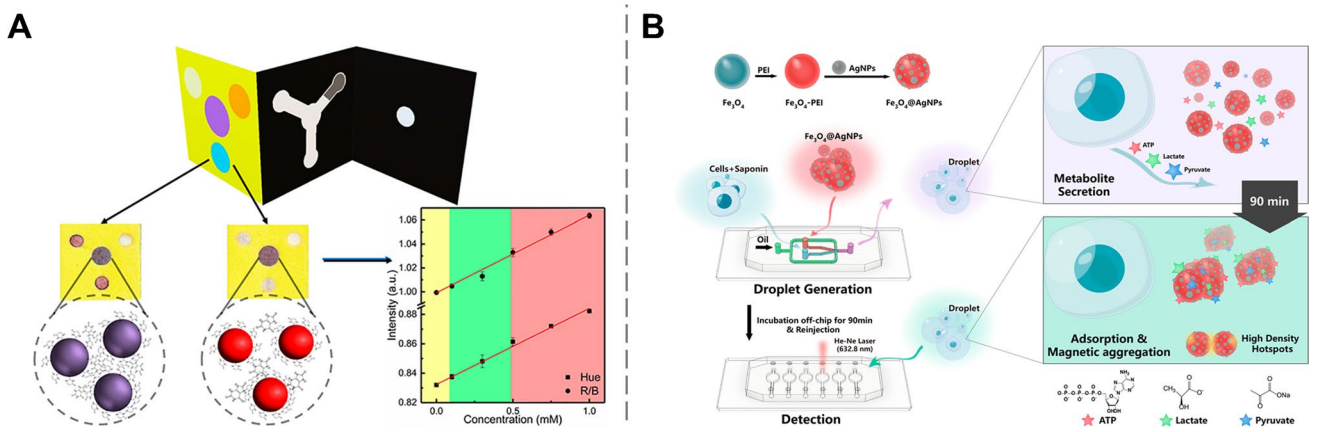


Fig. 6 Multiplexed detection of small molecules using nanomaterial-assisted microfluidic devices. (A) AuNP-based 3D paper microfluidic platform for colorimetric assays of glucose, uric acid, and free cholesterol. Reprinted with permission from [173]. Copyright 2021, American Chemical Society. (B) Encapsulation of AgNP-decorated Fe_3O_4 magnetic microspheres into droplets along with single cells for multiplexed detection of cellular metabolites. Reprinted with permission from [174]. Copyright 2019, American Chemical Society

was reported for the highly sensitive detection of glucose. CuNWs, displaying electrocatalytic activity toward carbohydrates, are considered excellent candidates for rapid and highly sensitive carbohydrate detection. CuNWs coupled with microfluidic chips were demonstrated to be successfully used as electrochemical sensors for the simultaneous detection of galactose 1-phosphate (Gal 1-P), galactose (Gal), and uridyl diphosphate galactose (UDP-Gal) in precious newborn urine samples for the diagnosis of type I, II, and III galactosemia diseases [176]. Magnetic nanoparticles can promote target separation and extraction, thus dramatically improving the detection sensitivity. A SERS-microfluidic droplet platform was developed where AgNP-decorated Fe_3O_4 magnetic nanocomposites along with one single cell were encapsulated into droplets for simultaneous measurement of single-cell secreted pyruvates, adenosine triphosphate (ATP), and lactate at low concentrations (Fig. 6B).

Nanomaterials in microfluidic devices could also enhance the signal readout. Silica nanoparticles were used in a μPAD to promote the adsorption of selected enzymes and prevent the washing away effect in the colorimetric measurements, thus yielding a dramatic improvement in color intensity and homogeneity. The platform succeeded in the simultaneous quantification of lactate, glucose, and glutamate in artificial urine samples [177]. A cerium metal–organic framework (Ce-MOF)-based origami paper SlipChip (OPSlipChip) was fabricated by our group [178], where the Ce-MOF nanoparticle significantly improved the uniformity and stability of the colorimetric readout. This nanoplatform was employed for highly selective detection of glucose and urine acid directly from human serum samples without any pretreatment, with limits of detection of 0.069 mM and 39.6 μM, respectively.

Applications

Nanomaterial-assisted microfluidics enables accurate, highly sensitive, and multiple detection of biomarkers (Table 1). By incorporating a specific detection apparatus for signal readout, these devices are applicable for analysis of complex biological or chemical samples in extensive areas, including disease diagnosis, environmental monitoring, food safety control, etc. Herein, we briefly introduce severa l aspects of their applications.

Disease diagnosis

Early diagnosis of diseases is of great significance, as it could offer prompt treatment and improve the prognosis of patients. Nanomaterial-assisted microfluidic technology combines the advantages of microfluidics and the particular properties of nanomaterials, which allows the simultaneous investigation of multiple biomarkers, minimization of sample quantity requirements, reduction of analysis time, and improvement of detection sensitivity and selectivity. This interdisciplinary technology shows tremendous potential in disease diagnosis [13].

One of the most frequent and important roles of using nanomaterials in microfluidic devices is the improvement of the detection sensitivity of rare cancer biomarkers. An immunoarray was proposed [122] based on the principle of ELISA with capture antibodies attached to the SWCNT-decorated electrode surface to enhance the capture efficiency of targets for the simultaneous detection of four prostate cancer biomarkers from 40 μL clinical serum samples, including prostate-specific antigen (PSA), prostate-specific membrane antigen (PSMA), platelet factor-4

Table 1 An overview of recent nanomaterial-assisted microfluidic methods for multiplexed detection of biomarkers

Microfluidic technique	Nanomaterial	Analytes	Role of the integrated-nanomaterial	Limit of Detection	Ref
Microarray	QDs	anti-IgG, anti-IgM, PSA	Multiplexity	PSA: 1 ng/mL	[140]
Microarray	SWCNTs, Silica NPs	IGF-1, PSA, PF-4, CD-14, VEGF-D, GOLM-1, PSMA, IGFBP-3	Sensitivity improvement	78 to 110 fg/mL	[151]
Microarray	Au@Ag NRs	human IgG, mouse IgG	Signal enhancement	10 fg/mL	[147]
Microarray	QDs	miRNA-21, miRNA-221, miRNA-16	Multiplexity	N.A.	[159]
Microarray	AuNPs	eight miRNA let-7 family members	Sensitivity improvement	0.2 to 0.8 pM	[166]
Microarray	SWCNT	PSA, PSMA, PF-4, IL-6	Sensitivity improvement	PSA: 1 ng/mL PSMA: 10 ng/mL PF-4: 1 ng/mL IL-6: 0.03 ng/mL	[122]
Microarray	AuNPs	PSA, IL-6	Sensitivity improvement	PSA: 0.23 pg/mL IL-6: 0.30 pg/mL	[179]
Microarray	SiNW	cTnT, CK-MM, CK-MB	Sensitivity improvement	1 pg/mL	[180]
Microarray	AuNRs	IL-2, IL-4, IL-6, IL-10, IFN-γ, TNF-α	Signal enhancement	6.46 to 20.56 pg/mL	[181]
Droplet microfluidics	QDs	miRNA-20a, miRNA-21, miRNA-155, miRNA-221	Multiplexity	35 to 39 pmol/L	[160]
Droplet microfluidics	MoS_2 nanosheets	HIV-1, HIV-2	Sensitivity improvement	HIV-1: 1.24 nM HIV-2: 1.26 nM	[165]
Droplet microfluidics	AgNPs	Pyruvates, ATP, lactate	Sensitivity improvement	N.A.	[174]
μPAD	AuNPs	FABP, cTnI, copeptin	Sensitivity improvement	FABP: 0.06 pg/mL cTnI: 0.3 pg/mL copeptin: 0.4 pg/mL	[146]
μPAD	ZnO NWs	FABP, cTnI, myoglobin	Signal enhancement	FABP: 1.36 ng/mL cTnI: 1.00 ng/mL myoglobin: 2.38 ng/mL	[149]
μPAD	GO, QDs	lysozyme, β-conglutin lupine	Sensitivity improvement	lysozyme: 343 ng/mL β-conglutin lupine: 2.5 ng/mL	[182]
μPAD	QDs, UCNP	uidA, Stx1A, tetA	Multiplexity, Signal enhancement	uidA: 26 fmol Stx1A: 56 fmol tetA: 76 fmol	[161]
μPAD	AuNPs	glucose, uric acid, free cholesterol	Multiplexity	glucose: 1.25 mM uric acid: 71 μM cholesterol: 81 μM	[173]
μPAD	Silica NPs	lactate, glucose, glutamate in artificial urine samples	Signal enhancement	lactate: 0.63 mM glucose: 0.50 mM glutamate: 0.25 mM	[177]
SlipChip	Ce-MOF NPs	gluocose, urine acide	Signal enhancement	glucose: 0.069 mM irine acide: 39.6 μM	[178]
Slip-driven device	PtNPs	CK-MB, troponin I, myoglobin	Sensitivity improvement	N.A.	[183]
Other microfluidics	CuNWs	Gal 1-P, Gal, UDP-Gal	Sensitivity improvement	Gal 1-P: 16 μM Gal: 15 μM UDP-Gal: 120 μM	[176]
Other microfluidics	AuNPs	AFP, PSA	Sensitivity improvement	500 pg/mL	[184]
Other microfluidics	GO	miR-125, miR-126, miR-191, miR-155, miR-21	Sensitivity improvement	0.146 aM	[185]
Other microfluidics	GO	Staphylococcus aureus, Salmonella enterica	Sensitivity improvement	11.0 CFU/mL	[186]

(PF-4), and interleukin-6 (IL-6), with limits of detection down to 1, 10, 1 and 0.03 ng/mL, respectively. A similar sandwich immunoarray platform was developed by the same group [179] by attaching capture antibodies strongly to the AuNP-modified electrode surface for the detection of PSA and IL-6 in the diluted serum of prostate cancer patients, with detection limits of 0.23 and 0.30 pg/mL, respectively. Another strategy is to functionalize the glass substrate of the PDMS microfluidic chip with AuNPs to provide LSPR and thus increase the detection sensitivity in such a way that the AuNPs are exactly located through the flow paths defined by the PDMS slab (Fig. 7A). This novel implementation was demonstrated to detect cancer-relevant biomarkers (human AFP and PSA) down to 500 pg/mL in a 50% diluted serum sample [184]. More recently, an attomole-level ultrasensitive microfluidic chip was reported (Fig. 7B), consisting of three-layer components, among which the glass substrate is functionalized with graphene oxide (GO) and poly-L-lysine (PLL) for more efficient miRNA trapping [185]. Five miRNAs (miR-125, miR-126, miR-191, miR-155, and miR-21) in real samples from breast cancer patients and healthy humans were simultaneously detected with an ultralow limit of detection. This platform showed excellent performance in clinical applications, especially for early cancer screening.

Apart from cancer diagnosis, nanomaterial-assisted microfluidics has also been applied for the diagnosis of other diseases or infections, with a reduction in sample consumption to a great extent. An integrated volumetric bar-chart chip (IV-Chip) was reported by using antibody-conjugated PtNPs as detection probes, where the PtNPs showed excellent catalytic activity to produce O_2 (readout signal) from H_2O_2 reduction [183]. The IV-Chip realized the multiplexed detection of myocardial infarction biomarkers creatine kinase (CK)-MB, troponin I, and myoglobin with only 1 µL of serum from finger-prick blood (Fig. 7C). A silicon nanowire (SiNW) array chip with SiNWs as sensitive sensors enabled the detection of three cardiac disease biomarkers, troponin T (cTnT), creatine kinase MM (CK-MM), and CK-MB, from a 2 µL fingerpick blood sample (Fig. 7D), with a low limit of detection of 1 pg/m [180]. Antibody-functionalized gold nanorods (AuNRs) were integrated into a microarray platform for parallel and quantitative measurement of multiple cytokines, e.g., bioactive proteins dynamically regulating the growth, maturation, and responsiveness of immune cells. Due to the enhancement of LSPR by the AuNRs, this microarray successfully monitored the inflammatory response in a 1 µL serum sample within 40 min of two infant patients with congenital heart defects after cardiopulmonary bypass surgery by measuring six different types of cytokines: interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-10 (IL-10), interferon-gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α) [181].

Environmental monitoring

Environmental monitoring is the observation and investigation of the environment and mainly includes soil monitoring, air monitoring, water monitoring, and waster monitoring. Because of its portable, robust, accurate, and fast analysis, nanomaterial-assisted microfluidic techniques make it possible to carry out in situ real-time environmental analysis that could reduce the cost, minimize the risk of contamination, and shorten the assay time by eliminating sample transportation [188, 189].

Soil pollution resulting from industrialization and urbanization has become a serious worldwide environmental problem that not only severely degrades soil fertility but also affects the whole ecosystem, since hazardous substances in soil may diffuse through water, air, plants, and animals, ultimately threatening human health. µPAD has been found in a wide variety of applications in the detection of soil contaminants such as phosphate, chlorate, explosive residues, heavy metals, etc. [61]. A special paper-based sensor printed with AgNPs to enhance the capture efficiency of airborne molecules was fabricated to probe trinitrotoluene (TNT) crystals and residues in an open environment. The TNT concentration in the soil was evaluated to be 1.4 ppm, with a detection limit of 1.6×10^{-17} g/cm^2 [190].

The contamination of water resources by heavy metals, pesticides, bacteria, pathogens, or even nuclear elements can have serious adverse health effects. Microfluidic devices have been widely used for water quality control [191–193]. A zigzag microfluidic chip integrated with AgNPs was proposed for the highly sensitive detection of As(III) ions [194] in local drinking water samples. A 3D origami µPAD combined with fluorescent QDs and molecularly imprinted polymers (MIPs) was developed to realize specific and sensitive detection of phycocyanin. QDs could act as fluorescent sensors by taking advantage of the quenched fluorescence of QDs by phycocyanin. Quantitative measurements were performed on both seawater and lake water samples, demonstrating the potential for water monitoring [195]. A similar technique was further employed by the same group to fabricate a 3D origami ion-imprinted µPAD integrated with QDs for simultaneous detection of Cu^{2+} and Hg^{2+} ions from seawater and lake water (Fig. 7E), where 0.07 µg/L Hg^{2+} and 0.32 µg/L Cu^{2+} in the lake water samples and 0.23 µg/L Hg^{2+} and 0.85 µg/L Cu^{2+} in the seawater samples were measured, satisfying the urban drinking water standard of these two ion concentrations [187]. These studies suggest new routines for water monitoring through nanomaterial-assisted microfluidic devices. AuNPs, having size-dependent colorimetric and fluorescent properties, can be highly sensitive sensors assisting microfluidic analytical devices for pollutant detection in groundwater samples [196]. This platform reached limits of detection down to 0.6 µg/L and

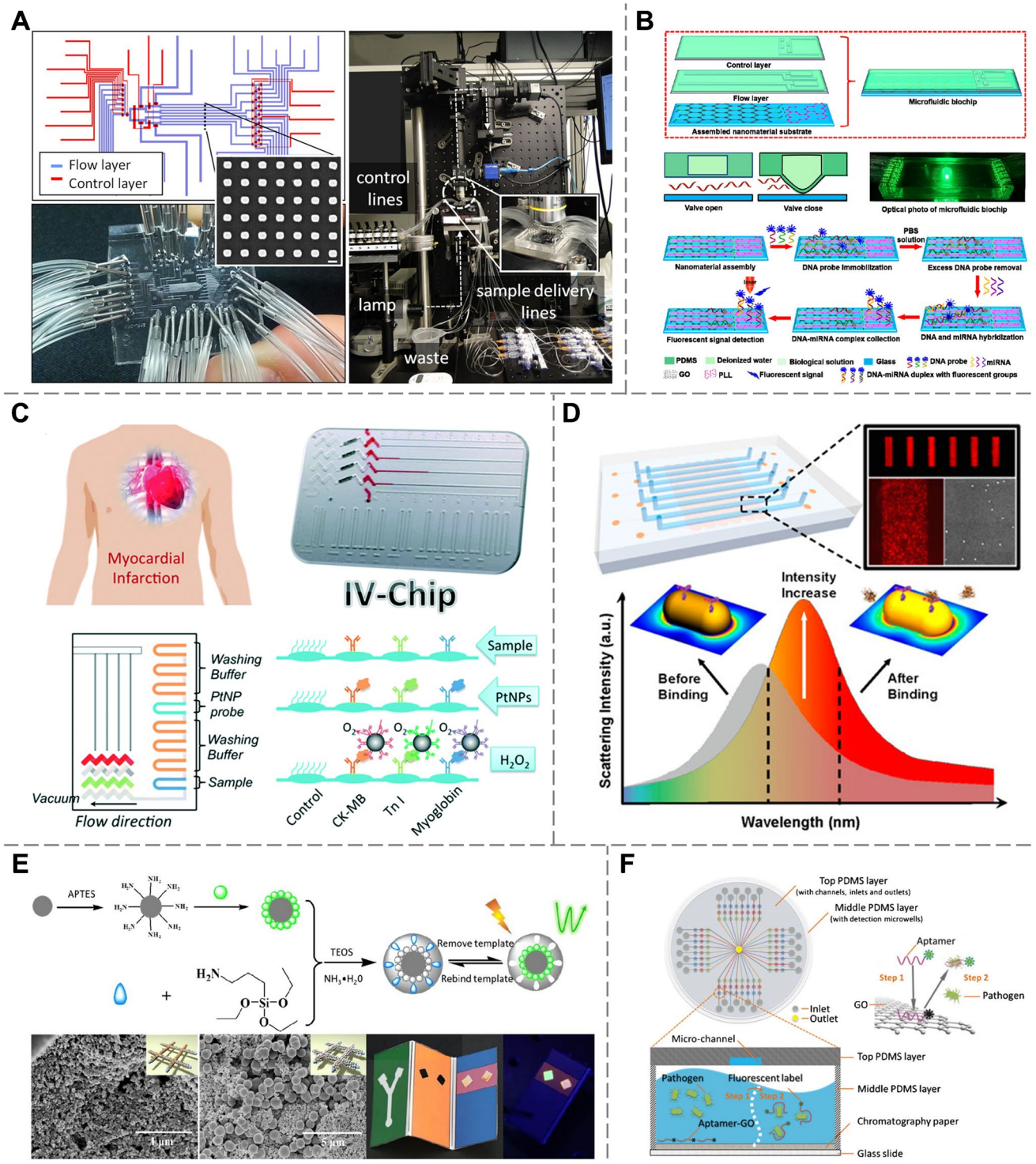


Fig. 7 Employment of nanomaterial-assisted microfluidics for the diagnosis of cancer and other diseases. (A) A microfluidic device integrated with AuNPs for cancer diagnosis. Reprinted with permission from [184]. Copyright 2014, American Chemical Society. (B) Biochip for multiple miRNA detection for breast cancer diagnosis. Reprinted with permission from [185]. Copyright 2021, American Chemical Society. (C) IV-Chip with antibody-conjugated PtNPs for myocardial infarction diagnosis. Reprinted with permission from [183]. Copyright 2016, Royal Society of Chemistry. (D) Antibody-functionalized AuNR microarrays for parallel measurement of multiple cytokines. Reprinted with permission from [181]. Copyright 2015, American Chemical Society. (E) 3D origami paper-based microfluidic chip for duplex detection of heavy metal ions. Reprinted with permission from [187]. Copyright 2017, Elsevier. (F) A hybrid microfluidic system integrated with fluorescently labeled aptamer-functionalized GO for multiple pathogen detection. Reprinted with permission from [186]. Copyright 2013, Royal Society of Chemistry

16 μg/L for the heavy metals Hg and dithiocarbamate pesticides, respectively.

Food safety control

Food safety monitoring is another application that can benefit greatly from nanomaterial-assisted microfluidics. Food safety has now attracted increasing attention since foodborne diseases caused by unsafe foods containing pathogenic bacteria, chemical contamination, allergens, etc., may not only influence individual health but also pose public health threats and economic problems [197, 198]. Traditional techniques, such as chromatography and spectroscopy, involve bulk instrumentations and are not suitable for rapid POCT. Highly integrated, miniaturized nanomaterial-integrated microfluidic devices have emerged as promising approaches to realize fast, cost-effective, efficient food safety control.

Pathogenic bacteria, mainly concerning Escherichia coli, Salmonella, Campylobacter, Listeria monocytogenes, and Clostridium perfringens, which can cause the majority of foodborne diseases, is the most common and severe food safety problem. Nanomaterial-assisted microfluidics provides a new routine to detect foodborne pathogens in a faster and more sensitive manner. A microfluidic chip integrated with magnetic beads for target separation and antibody-conjugated QDs for fluorescent labeling was proposed for Salmonella typhimurium detection in chicken samples with a sensitivity of 103 CFU/mL [199]. Zuo et al. [186] reported a microfluidic system integrated with graphene oxide (GO) functionalized with fluorescently labeled aptamers. GO has a reversible fluorescence quenching property when the fluorescently labeled aptamers are adsorbed or desorbed, thus can serve as a sensitive fluorescence sensor for multiplexed pathogen detection (Fig. 7F). The device simultaneously detected Staphylococcus aureus and Salmonella enterica, reaching a detection limit of 11.0 CFU/mL, displaying great potential for extensive application in the detection of other bacterial and viral pathogens in food.

Heavy metal contamination in food may cause damage to neurological and kidney tissues, hence is very dangerous to human health. Similarly, fluorescently labeled DNA-functionalized GO holds the role of fluorescent sensor and was integrated into a μPAD to simultaneously detect different chemical contaminants in food, including the heavy metal Hg^{2+}, Ag^{+}, and aminoglycoside antibiotic residues in food. This low-cost and simple device offers attractive applications in food safety control [60].

Food allergies are another food safety problem that should be considered. It is of great importance to detect allergens in food to avoid food for allergic individuals. A microfluidic platform integrated with QDs-aptamer-functionalized GO as a fluorescent sensor was developed to detect the peanut allergen Ara h1 and reached high detection sensitivity and selectivity [200]. Later, the same group fabricated a μPAD by using the same fundamental to realize the detection of egg allergens white lysozyme and β-conglutin lupine within 5 min [182].

Other food contaminants, including pesticide residues, biotoxins, antibiotics, harmful additives, etc., may also threaten the health or life of humans. An electrochemical microfluidic chip was integrated with GO-supported AuNPs for signal amplification, and the device was reported to determine the insecticide carbofuran with improved sensitivity, achieving a limit of detection down to 67 pM [201]. An integrated microfluidic chip based on a smectite-polyacrylamide nanocomposite was developed for the quantitative detection of aflatoxin in corn samples, and a very low limit of detection, at the sub100 ppb level, was achieved [202].

Summary and outlook

Capable of precisely controlling small volumes of liquids, microfluidics has emerged as an ideal platform for biochemical analysis, exhibiting advantages such as parallelization, high throughput, minimal sample consumption, low cost, and short reaction time. In addition, modern nanofabrication technology ensured the integration of complicated functions into microfluidic devices, enlarging the employment of microfluidic techniques toward the development of highly integrated POCT devices. Nanomaterials possess unique characteristics, including high surface-to-volume ratio, easy functionalization, intrinsic catalytic activity, enhanced surface plasmon resonance, and tuned optical properties, making them outstanding candidates for biological analysis. Nanomaterial-assisted microfluidics benefitting from the advantages of both components, enables multiplexed detection of biomarkers using a minimum amount of sample with increased sensitivity, high specificity, low detection limit, intensive signals, short detection time, etc. Compared with other traditional or commercial techniques such as lateral flow assays (LFA) and mass spectrometry (MS), these techniques show great capability to overcome the challenges in biomarker analysis in complex biological samples, including small quantities of available samples, low concentration of target molecules and long reaction time for detecting multiple analytes. Extensive applications have been reported in disease diagnostics by the assessment of biomarkers for cancer or other diseases, in environmental monitoring through the detection of contaminants, heavy metals or organic pollutants in water and soil, and in food safety control via the evaluation of harmful substances such as pathogenic bacteria, heavy metals, allergens, and toxins.

Nevertheless, there is still a long way for these innovative and powerful devices to be commercialized and universally

applied in future POCT and clinical diagnostics. First, since most of the outstanding properties of nanomaterials highly depend on their sizes and shapes, the reproducible synthesis of abundant nanoparticles with consistent properties satisfying clinical requirements remains a major challenge. Then, the currently reported nanomaterial-assisted microfluidic techniques are laboratory concepts for fundamental research, far from the large-scale commercialization for clinical applications. Therefore, global standardization of the microfluidic devices is indispensable for a more extensive implementation. Finally, it is challenging to fabricate miniaturized, automatic, and easy-to-handle nanomaterial-assisted microfluidic devices with convenient operation, fast reaction, and straightforward signal readout, suitable for clinical diagnostics and quotidian use.

Going forward, the field needs to continue to evolve along with nanomaterial engineering in terms of reproducible synthesis, functionalization, new nanomaterial design, etc., and microfabrication technology in terms of nanomaterial integration, standardized fabrication, novel architecture design, etc., to realize compact, integrated, miniaturized, and east-to-use devices for portable analysis. Nanomaterial-assisted microfluidics has been incredibly successful in recent years, and it can be reasonably expected that, in the near future, numerous nanomaterial-assisted microfluidic devices will be found in laboratories, hospitals, food factories, nursing homes, and even at home.

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Declarations

Conflict of interest The authors declare no competing interests.

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